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# SYSTEMATICS OF *PRESTONIA* (APOCYNACEAE: APOCYNOIDS: ECHITEAE) 80 YEARS AFTER WOODSON[1,2]

*J. Francisco Morales,*[3,4] *Mary E. Endress,*[5] *and Sigrid Liede-Schumann*[3]

Abstract

*Prestonia* R. Br. (apocynoids, Echiteae) comprises ca. 58 species, which are lianas with eglandular leaves, axillary or terminal cymose inflorescences, sepals with a single colleter, flowers usually with an annular corona around the mouth and/or free corona lobes, follicular fruits, and truncate seeds that are comose at the micropylar end. Three infrageneric classifications have been proposed in the last 110 years, all of them based only on morphological characters and none of which have been tested with molecular data. In the present study, based on the analyses of 62 nuclear and 258 chloroplast sequences from 65 species, we test the monophyly of *Prestonia* as well as the infrageneric classifications proposed for the genus. *Prestonia* was shown to be monophyletic, but the infrageneric sections proposed by Schumann, Woodson, and Pichon are all non-monophyletic. We propose a new infrageneric classification for *Prestonia*, recognizing six sections: *Coalitae* Woodson, *Denticulatae* J. F. Morales, M. E. Endress & Liede, *Exsertae* J. F. Morales, M. E. Endress & Liede, *Haemadictyon* (Lindl.) Baill., *Mollis* J. F. Morales, M. E. Endress & Liede, and *Prestonia*. A key to the sections is provided. *Prestonia pickelii* Markgr. is removed from the synonymy of *P. quinquangularis* (Jacq.) Spreng. and resurrected as an accepted species. We provide a brief overview of the prolific groundbreaking work of Robert E. Woodson and Marcel Pichon in the Apocynaceae and *Prestonia*, and respectfully pay homage to their invaluable contributions to our knowledge of the family.

*Key words:* Apocynoids, classification, ITS, molecular phylogeny, Prestoniinae, systematics.

Robert E. Woodson, Jr., at the Missouri Botanical Garden in St. Louis, and Marcel Pichon, at the Muséum National d'Histoire Naturelle in Paris, were two of the most important specialists of Apocynaceae in the first half of the 20th century. Both published a large number of key papers and monographic works that greatly furthered knowledge on Apocynaceae, particularly the rauvolfioids and apocynoids (Humbert & Léandri, 1955; Allen et al., 1965; Nevling, Jr., 1965). They both possessed a keen eye and an innate feel for "good" characters. Thus,

[1] We dedicate this work to the memory of Robert E. Woodson, Jr., to commemorate the 80th anniversary of his landmark monograph of the Neotropical apocynoids published in the *Annals of the Missouri Botanical Garden* in 1936.

[2] Xavier Cornejo (GUAY) and Alessandro Rapini (HUESF) sent preserved material of *Prestonia amabilis* and *P. bahiensis*, respectively; Xavier also provided several photos and notes of species of *Prestonia* from Ecuador; their help is gratefully acknowledged. We thank Paul Forster (BRI) for generously sending leaf samples of several species of *Parsonsia*. Barry and Isa Hammel (MO) provided several DNA samples of species from Costa Rica; their support in the last four years is greatly appreciated. Bruce Hansen (USF) generously supported the visit of the first author to the University of South Florida. We extend our thanks to the following herbaria, which allowed the study of their collections: B, C, CEPEC, CICY, COAH, COL, CR (including INB), E, F, G, GUAY, HUA, HUQ, JAR, JAUM, L, M, MBML, MEDEL, MHES, MO, NY, O, P, PMA, RB, S, U, UBT, US, USF, USJ, WAG, and Z. Angelika Täuber and Margit Gebauer from Plant Systematics Molecular Laboratory, Universität Bayreuth, Germany, assisted and helped during the DNA extraction and amplification processes. The first author thanks Michael Grayum (MO) for his continued support in many ways, providing critical references and the proper interpretation of the International Code of Botanical Nomenclature. We gratefully thank Andre Simões (University of Campinas, Brazil) and Rafael Acuña (University of Costa Rica, Costa Rica) for kindly sharing their original and modified *matK* amplification protocols. This study was supported by a Ph.D. research grant (Forschungsstipendien für Doktoranden und Nachwuchswissenschaftler für mehr als 6 Monate) from the Deutscher Akademischer Austauschdienst (DAAD) to J. Francisco Morales, which is gratefully acknowledged. Financial support was provided by the University of Bayreuth Graduate School (Germany) and the Deutscher Akademischer Austauschdienst (Germany). The first author wishes to express his gratitude to the Missouri Botanical Garden, in particular, Olga Martha Montiel, who have supported several visits to their herbarium over the last decade. The first author thanks the following persons for field assistance: Alfredo Fuentes, Alev Oder, and Daniel Soto (Bolivia); Marccus Alves, Diogo Araujo, Andre Fontana, Ludovic Kollmann, Isa Lucia de Morais, Ricardo Perdiz, and Alessandro Rapini (Brazil); Fernando Alzate, Julio Betancur, Ricardo Callejas, Dayron Cárdenas, Felipe Cardona, Álvaro Idárraga, and Mario Alberto Quijano (Colombia); Ronald Abarca, Isler Chinchilla, Frank González, and Luis Fonseca (Costa Rica); Gabriel Cerén, Eunice Echeverría, and Jenny Menjivar (El Salvador); Carlos Rocha (Nicaragua); Cármen Galdámez (Panama); Carlos Amasifuen, Aniceto Daza, and Carlos Reynel (Peru); and Ronald Abarca and Carlos González (Venezuela).

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despite their work being based exclusively on morphological characters, which are notoriously prone to repeated evolutionary trends in distant clades, their great knowledge of the plants allowed them to reach often surprisingly accurate hypotheses of relationship in difficult groups. Until the advent of molecular phylogenetics, their treatments provided the framework for our understanding of the family and into which new species were continually being intercalated. The two were also good friends. For example, Woodson said about his new species *Aspidosperma pichonianum* Woodson, "The species commemorates my friend M. Marcel Pichon, of the Muséum National d'Histoire Naturelle" (Woodson, 1951: 176). And, both died suddenly and tragically young in the midst of their careers: Woodson was 59 and Pichon only 33 years old, leaving a huge gap in the research of one of 10 largest families of flowering plants (Rapini, 2012).

As a curator at the Missouri Botanical Garden (Mathias, 1965), with its intensive collecting in Latin America, Woodson was in an ideal position to study the many specimens of Apocynaceae pouring in from collectors in the Neotropics. In order to put names on them, he first had to come to terms with *Echites* P. Browne. One must keep in mind that in the 19th century, more or less all Neotropical apocynoid lianas were described in the genus *Echites*. It was Woodson who is responsible for the seemingly Herculean task of painstakingly sorting through the descriptions and scrutinizing the types of all Neotropical plants described as *Echites*, describing new genera as needed, resolutely bringing order out of chaos. He also tackled the confusion brought about by John Miers, who was not only a consummate splitter, but at the same time combined unrelated taxa (Morales et al., 2007b; Simões et al., 2010), teasing apart the conglomerate "genera," gathering like with like and redefining them. Many of the current generic circumscriptions used in Neotropical taxonomy are those proposed by Woodson (e.g., Woodson, 1931, 1932a, 1932b) and especially his large monograph of Neotropical apocynoids (Woodson, 1933, 1934, 1935, 1936). Woodson published the last monograph of *Prestonia* (Woodson, 1936). In that work and subsequent publications, he described 30 new species and transferred another 14 from other genera, making him the author of 44 species, and thus the most prolific systematist who ever worked in *Prestonia*.

Pichon, as head of phanerogams at the Museum National d'Histoire Naturelle in Paris, focused mainly on the big picture: classification at the genus level and above and on a global scale. He was amazingly productive in his short life, publishing a prodigious number of large, broad-based worldwide classifications down to subtribal level, incorporating detailed morphological descriptions of floral organs (e.g., Pichon, 1948b), as well as a number of smaller papers, especially to improve the infrageneric classification of several larger genera in both rauvolfioids and apocynoids (e.g., Pichon 1947a, 1947b, 1948a, 1948c, 1950a, 1950b, 1950c). For his outstanding monograph of Landolphieae in Africa (Pichon, 1953), he was awarded the Prix De Candolle in 1953 (Humbert & Léandri, 1955). He provided the last infrageneric classification of *Prestonia* (Pichon, 1950a), placing a few species overlooked by Woodson and intercalating species described after publication of Woodson's monograph (Woodson, 1936).

## Classification of Apocynaceae Today

Apocynaceae includes more than 366 genera and 5000 species (Rapini, 2012) and is distributed throughout the tropics, subtropics, and temperate regions. Endress and Bruyns (2000) recognized five subfamilies: Rauvolfioideae and Apocynoideae from the traditional Apocynaceae, and Periplocoideae, Secamonoideae, and Asclepiadoideae from the traditional Asclepiadaceae. Since then a number of phylogenetic studies have contributed to the resolution of relationships at the tribal, subtribal, or generic level, which has led to the reorganization or description of tribes and subtribes and the synonymization of several genera (e.g., Meve & Liede, 2004; Simões et al., 2004, 2006, 2010; Liede-Schumann et al., 2005; Rapini et al., 2006, 2011; Endress & Hansen, 2007; Endress et al., 2007a, 2007b; Livshultz et al., 2007; Silva et al., 2012; Khanum et al., 2016; Meve et al., 2017; Morales et al., 2017). Despite these advances, many groups are still unresolved or the resolution is too low to accurately define their relationships. Apocynoideae and Rauvolfioideae remained paraphyletic, and thus informal ranks were proposed for these two subfamilies by Simões et al. (2016). The family thus currently comprises three formal subfamilies (Periplocoideae, Secamonoideae, and Asclepiadoideae) and two informal groups (apocynoids and rauvolfioids), the classification followed in Morales et al. (2017) and here.

In the apocynoids, nine tribes were proposed by Endress et al. (2014): Apocyneae, Baisseeae, Echiteae, Malouetieae, Mesechiteae, Nerieae, Odontadenieae, Rhabdadenieae, and Wrightieae, of which only Echiteae, Mesechiteae, and Rhabdadenieae (the last comprising only one genus) have been resolved as monophyletic (Simões et al., 2004; Livshultz et al., 2007; Livshultz, 2010; Morales et al., 2017). Echiteae included 19 genera divided among five subtribes: Echitinae, Parsonsiinae, Peltastinae, Pentalinoninae, and Prestoniinae. A molecular phylogenetic evaluation by Morales et al. (2017) found that the tribe and all its subtribes, as circumscribed by Endress et al. (2014), were paraphyletic. One subtribe (Pentalinoninae) was excluded because it came out with the outgroup; and the remaining four subtribes were recircumscribed; and a new tribe (Laubertiinae) was described; in addition, two genera (*Fernaldia* Woodson and *Peltastes* Woodson) were found to be paraphyletic

and were reduced to synonyms. In the classification of Morales et al. (2017), Echiteae comprises 14 genera and ca. 200 species, distributed among five subtribes: Echitinae, Laubertiinae, Parsonsiinae, Peltastinae, and Prestoniinae. *Parsonsia* R. Br. (Parsonsiinae, 80 to 90 species) and *Prestonia* R. Br. (Prestoniinae, ca. 58 species) are by far the largest genera in the tribe, whereas the remaining genera all contain less than 15 species.

## The Genus *Prestonia*

*Prestonia* is distributed in the Neotropics, ranging from Mexico and the West Indies to northern Argentina in a wide variety of habitats, from sea level up to 2800 m. The genus has a complex taxonomic history. It was described by Brown (1810) based on a single species (*P. tomentosa* R. Br.), characterized by having corollas with an annular corona around the mouth and five free corona lobes within the tube. Lindley (1826) proposed *Haemadictyon* Lindl., which he distinguished from *Prestonia* by its nectary composed of five individual lobes (vs. an annular disc nectary). De Candolle (1844) recognized *Prestonia* and *Haemadictyon* as distinct genera, describing some new species and transferred some others to other genera. Miers (1878) was the first to reduce *Haemadictyon* to a synonym of *Prestonia*, but at the same time, he described other new genera such as *Mitozus* Miers, *Rhaptocarpus* Miers, and *Temnadenia* Miers, to which he transferred several species of *Prestonia* together with species of unrelated "good genera," creating taxonomic havoc that prevailed for more than 50 years. Baillon (1891) and Schumann (1895) also considered *Haemadictyon* to be a synonym of *Prestonia*. Schumann made some additional combinations but he did not evaluate the genera proposed by Miers (1878), and thus, the taxonomy of *Prestonia* remained confused. The monotypic *Belandra* S. F. Blake was described based on a single specimen from Belize and assumed to be related to *Echites* and *Odontadenia* Benth., but without discussion of its relationships with *Prestonia* (Blake, 1917). Woodson (1931, 1936) made a significant contribution to clarifying the taxonomy of *Prestonia* by sorting out the nomenclatural mess created by Miers (1878), thereby including *Rhaptocarpus* wholly, and *Mitozus* and *Temnadenia* in part, as synonyms. He also reduced *Belandra* to a synonym and described many new species. His circumscription of *Prestonia* recognized 60 species distributed in four sections (Woodson, 1936). Pichon (1950a) recognized five sections and accepted 66 species, including some species described after the publication of Woodson's classification in 1936 (e.g., *P. dentigera* Woodson, *P. macrophylla* Woodson, *P. pickelii* Markgr.) and proposed a new combination for *Haemadictyon cayennense* A. DC. (*P. cayennensis* (A. DC.) Pichon), which was overlooked by Woodson (1936).

*Prestonia mollis* Kunth and *P. quinquangularis* (Jacq.) Spreng. exhibit a high degree of morphological variation, in vegetative as well as floral parts, which has led to the description of a number of species over the years. Woodson (1936) described *P. cordifolia* Woodson, which he included in *Prestonia* sect. *Tomentosae* based on its pubescent corolla and leaves and purple flowers. However, the type is similar to some extreme morphotypes of *P. mollis* observed by the first author in the Peruvian Andes. In the same work, Woodson accepted several species (*P. acutifolia* (Benth. ex Müll. Arg.) K. Schum., *P. marginata* (Benth.) Woodson, *P. pachyphylla* Woodson, and *P. simulans* Woodson), which are morphologically similar to *P. quinquangularis*, and which he differentiated from the last only by their coriaceous leaf blades, a character sometimes present in specimens from the Amazon Basin or the Guiana Shield. All these taxa have been reduced to the synonymy of *P. quinquangularis* during the past decade (e.g., Morales, 2006, 2007b, 2010). However, the name *P. marginata* has been involved in a confused nomenclatural situation. Bentham (1841) proposed *Haemadictyon marginatum* Benth., a species from southwestern Guyana (Upper Takutu-Essequibo, Pirara). Markgraf (1924) described *P. marginata* based on a Ule specimen from Roraima State, Brazil. Both type collections are conspecific and were collected in the Guiana Shield. Woodson (1936) considered *Haemadictyon* a synonym of *Prestonia* and made the combination *P. marginata* (Benth.) Woodson based on Bentham's name. At the same time, he erroneously considered Markgraf's name (1924) to be illegitimate and proposed a new name, *P. pachyphylla*, for that species. However, the name by Markgraf (1924) has priority according to Article 11.4 of the International Code of Nomenclature (McNeill et al., 2012), and thus, Woodson's names *P. marginata* (Benth.) Woodson and *P. pachyphylla* are both illegitimate. The placement of *P. marginata* Markgr. has been in dispute. Morales (2007b) reduced it into the synonymy of *P. quinquangularis*, but in some checklists and floristic treatments for South America (e.g., Zarucchi et al., 1995; Funk et al., 2007) it has been placed under the synonymy of *P. cayennense* (A. DC.) Pichon.

*Prestonia* has continued to be embroiled in disputes regarding its generic circumscription for the last 80 years. Woodson (1931, 1936) transferred *Echites agglutinatus* Jacq. to *Prestonia*; he included *P. agglutinata* (Jacq.) Woodson in *Prestonia* sect. *Coalitae* Woodson, despite its lack of either an annular corona around the mouth or free coronal lobes within the tube. Woodson (1960) described *P. caudata* Woodson, a species with some shared morphological characters with *P. agglutinata* (Jacq.) Woodson, including lack of an annular corona and free corona lobes, which he thus also included in *Prestonia* sect. *Coalitae*. Gentry (1983) transferred *E. woodsonianus* Monach. to *Prestonia*, based on the

acceptance of *P. agglutinata*. Morales (1997b) evaluated *Prestonia* sect. *Coalitae*, and based on floral morphological characters, determined that *P. agglutinata*, *P. caudata*, and *P. woodsoniana* (Monach.) A. H. Gentry were inappropriately placed in *Prestonia*, and transferred them to *Echites*. In a cladistic analysis based on morphology alone, these three species grouped together in a clade separate from the included species of *Echites* (Williams, 2004) and were thus treated as a separate genus, *Allotoonia* J. F. Morales & J. K. Williams (Morales & Williams, 2004). In the study by Livshultz et al. (2007) the two included species of *Allotoonia* were nested in a clade with the included species of *Echites* and *Fernaldia*. This relationship was corroborated by Morales et al. (2017), who treated both *Allotoonia* and *Fernaldia* as synonyms of *Echites*.

*Rhodocalyx* Müll. Arg., a genus of erect herbs from the cerrados of southern South America, was reduced to the synonymy of *Prestonia* by Morales (1999) based on the presence of an annular corona around the corolla mouth. In a molecular phylogenetic study of tribe Mesechiteae by Simões et al. (2004), *Rhodocalyx* was included as part of the outgroup and was retrieved together with *P. riedelii* (Müll. Arg.) Markgr., a relationship that was confirmed by Livshultz et al. (2007), suggesting the paraphyly of *Prestonia*. *Rhodocalyx* was, therefore, reinstated as a valid genus by Livshultz et al. (2007). In the study by Morales et al. (2017), *P. riedelii* was again resolved as sister to *Rhodocalyx* and this clade was far removed from the other included species of *Prestonia*; *P. riedelii* was thus transferred to *Rhodocalyx*. *Temnadenia* also has been involved with *Prestonia*. The last revision recognized four species (Morales, 2005b) and three of them were included in the study by Morales et al. (2017), one of which, *T. ornata* (Hoehne) Woodson, was deeply nested among species of *Prestonia* and was therefore transferred to that genus.

Four infrageneric classifications have been proposed for *Prestonia*. The first was that of Baillon (1891), who recognized three sections (*Prestonia* sect. *Euprestonia* Baill., *Prestonia* sect. *Prestonianthe* Baill., and *Prestonia* sect. *Haemadictyon* (Lindl.) Baill.). The last is based on *Haemadictyon* Lindl., but since he did not cite the species included in the other two sections, his proposal cannot be properly evaluated. The name *Prestonia* sect. *Euprestonia* is a nom. inval. according to Article 21.3 of the Code (McNeill et al., 2012). Of the remaining three classifications (Table 1), the first, by Schumann (1895), recognized two sections: *Prestonia* sect. *Euprestonia* Baill. (seven species) and *Prestonia* sect. *Haemadictyon* (Lindl.) K. Schum. (three species), based on pubescence of stems, inflorescence type, and sepal shape. Schumann's section name *Prestonia* sect. *Haemadictyon* is illegitimate because it was based on the same type as *Prestonia* sect. *Haemadictyon* (Lindl.) Baill., and *Prestonia* sect. *Euprestonia* is invalid (Art. 21.3; McNeill et al., 2012). The second classification, by Woodson (1936), recognized four sections: *Prestonia* sect. *Acutifoliae* Woodson, *Prestonia* sect. *Annulares* Woodson, *Prestonia* sect. *Coalitae*, and *Prestonia* sect. *Tomentosae* Woodson, which were differentiated based on degree of corolla tube pubescence, shape and texture of the sepals, and characteristics of the annular corona, free corona lobes, and anthers. The largest section, *Prestonia* sect. *Annulares*, included 33 species, *Prestonia* sect. *Tomentosae* with 14 species, *Prestonia* sect. *Acutifoliae* with nine species, and *Prestonia* sect. *Coalitae*, the smallest, with only four species. The section names *Acutifoliae* and *Tomentosae* were illegitimate, because they included the types of Baillon's *Presontia* sect. *Haemadictyon* and Schumann's *Prestonia* sect. *Euprestonia*, respectively. The most recent infrageneric classification of *Prestonia* was proposed by Pichon (1950a), who recognized five sections, based on length of the corolla lobes, type of suprastaminal indumentum within the corolla tube, and features of the gynoecium and androecium. He accepted Schumann's sections *Haemadictyon* (with 50 species, including in the synonymy Woodson's sections *Annulares* and *Acutifoliae*) and *Prestonia* sect. *Euprestonia* (with 11 species, a nom. inval. according to Art. 21.3 of the Code; McNeill et al., 2012), but not Woodson's *Prestonia* sect. *Coalitae*, the members of which he placed in two different sections: *Prestonia* sect. *Rhaptocarpus* Pichon (three species) and *Prestonia* sect. *Trichopharynx* Pichon (one species). He also proposed section *Tetraceras* based on *P. parviflora* (Benth.) Benth. & Hook. f. *Rhaptocarpus* is an illegitimate name, because it includes the type of Woodon's *Prestonia* sect. *Coalitae* (Woodson, 1936), whereas the type of Pichon's fourth section, *Prestonia* sect. *Trichopharynx* Pichon, was transferred to *Echites* by Morales (1997b). These three infrageneric classification systems differ as to the composition of each section, have nomenclatural problems, were based only on morphological characters, and have never been evaluated using molecular data. New species have been described by several authors over the years, and many others have been reduced to synonymy (e.g., Woodson, 1939, 1948; Woodson & Schery, 1940, 1942; Gentry, 1974; Markgraf, 1975; Morales, 1996, 1997a, 2004a, 2004b, 2004c, 2006, 2007a, 2007b, 2010, 2011; Morales & Liede-Schumann, 2016), adding to the current infrageneric confusion.

## Objectives

The use of markers from nuclear ribosomal DNA (nrDNA) has been shown to be informative and has helped resolve relationships between species and genera (e.g., Baldwin et al., 1995; Álvarez & Wendel,

Table 1. Sections of *Prestonia* R. Br. in three infrageneric classifications.

| | Total accepted species | Section | Number of species |
|---|---|---|---|
| Schumann (1895) | 10 | *Euprestonia* Baill., nom. inval. | 7 |
| | | *Haemadictyon* (Lindl.) K. Schum., nom. superfl. | 3 |
| Woodson (1936) | 60 | *Acutifoliae* Woodson, nom. illeg. | 9 |
| | | *Annulares* Woodson | 33 |
| | | *Coalitae* Woodson | 4 |
| | | *Tomentosae* Woodson, nom. illeg. | 14 |
| Pichon (1950a) | 66 | *Euprestonia* Baill., nom. inval. | 11 |
| | | *Haemadictyon* (Lindl.) Baill. | 50 |
| | | *Rhaptocarpus* Pichon, nom. illeg. | 3 |
| | | *Tetraceras* Pichon | 1 |
| | | *Trichopharynx* Pichon | 1 |

2003), even species complexes that have been particularly difficult to resolve with chloroplast DNA (cpDNA), due to a rapid or recent radiation (Álvarez & Wendel, 2003; Bell et al., 2015). In Apocynaceae, the use of nuclear markers has helped to resolve or elucidate some problematic groups in Periplocoideae and Asclepiadoideae (e.g., Meve & Liede, 2001; Goyder et al., 2007; Ionta & Judd, 2007; Surveswaran et al., 2009; Bruyns et al., 2010; Wanntorp et al., 2014; Joubert et al., 2016; Khanum et al., 2016). But in apocynoids and rauvolfoids, the use of nuclear markers has been limited and mostly restricted to Old World groups (e.g., Hendrian & Kondo 2007a, 2007b, 2007c; Middleton & Livshultz, 2012; Uemachi & Shimomura, 2013). Only three studies (utilizing either phytochrome A and internal transcribed spacer [ITS]) have been published that included more than a handful of Neotropical genera of apocynoids and/or rauvolfioids: Potgieter (1999), Livshultz (2010), and Morales et al. (2017). The infrageneric relationships of larger genera often remain unclear or are totally unresolved, and the monophyly of several earlier infrageneric classifications based only on morphological characters has not been tested.

Although significant progress has been made in our understanding of relationships within Echiteae in the past decade, rarely have more than one or two species of *Prestonia* been included in molecular-based studies. Eight species were included by Livshultz et al. (2007) in a study using cpDNA. In a phylogenetic study of Echiteae based on nrDNA and cpDNA markers by Morales et al. (2017), 16 of the 56 currently recognized species of *Prestonia* were included. In that paper, the monophyly of *Prestonia* was hypothesized, after inclusion of *Temnadenia ornata* as a new combination and transfer of *P. riedelii* to *Rhodocalyx*. But a larger sample is needed to accurately test monophyly of the genus and to evaluate the current infrageneric classification proposals (Schumann, 1895; Woodson, 1936; Pichon, 1950a). Here we present a phylogenetic analysis of *Prestonia* based on sequences from four cpDNA regions (*matK* + *5′/3′ trnK* intron, *rpl16* intron, *rps16* intron, and *trnL* intron + *trnL-trnF* intergenic spacer) and one nrDNA region, the ITS. The sampling includes ca. 86% of the currently accepted species (50/ca. 58 species, Morales & Liede-Schumann, 2016). Our aims are fivefold: (1) to test the monophyly of *Prestonia* with a larger sample than in previous studies; (2) to recover the relationships among species of *Prestonia*; (3) to evaluate the infrageneric classifications of Schumann (1895), Woodson (1936), and Pichon (1950a); (4) to provide a new infrageneric classification, including taxonomic realignments and nomenclatural changes as needed; and (5) to clarify the status of some disputed species like *P. mollis–P. cordifolia* and *P. quinquangularis–P. marginata*.

## Materials and Methods

### Taxon sampling

Fifty of the 58 currently recognized species of *Prestonia* were sampled, including at least two members each from all sections of Schumann (1895), Woodson (1936), and Pichon (1950a) (Table 2). The classification of Schumann (1895) included 10 species, of which nowadays only three are accepted, the other seven being treated as synonyms. The outgroup taxa included 13 species from six genera of Echiteae (*Artia* Guillaumin, *Asketanthera* Woodson, *Echites*, *Laubertia* A. DC., *Parsonsia*, and *Temnadenia*) representing subtribes Echitineae, Parsonsiinae, and Peltastinae, which were shown to be the groups most closely related to *Prestonia* (Morales et al., 2017). In addition, two species of *Odontadenia* (Odontadeniae) were used as rooting taxa.

In order to determine the relationship of *Prestonia mollis–P. cordifolia* and *P. quinquangularis–P. marginata*, two samples each of *P. mollis* and *P. quinquangularis* were included: one of the typical morphotype of both

Table 2. Species of *Prestonia* R. Br. and their sectional placement in three previous infrageneric classifications and this study.

| | Schumann (1895) | Woodson (1936) | Pichon (1950a) | This study |
|---|---|---|---|---|
| *P. acrensis* J. F. Morales | – | – | – | *Prestonia* |
| *P. acutifolia* (Benth. ex Müll. Arg.) K. Schum. | *Haemadictyon* | *Acutifoliae* | *Haemadictyon* | * |
| *P. agglutinata* (Jacq.) Woodson | – | *Coalitae* | *Trichopharynx* | ** |
| *P. amabilis* J. F. Morales | – | – | – | *Denticulatae* |
| *P. amanuensis* Woodson | – | *Tomentosae* | *Euprestonia* | * |
| *P. amazonica* (Benth. ex Müll. Arg.) J. F. Macbr. | – | *Annulares* | *Haemadictyon* | *Denticulatae* |
| *P. annularis* (L. f.) G. Don | – | *Annulares* | *Haemadictyon* | *Haemadictyon* |
| *P. antioquiana* J. F. Morales & Liede | – | – | – | *Denticulatae* |
| *P. bahiensis* Müll. Arg. | – | *Tomentosae* | *Euprestonia* | *Prestonia* |
| *P. boliviana* J. F. Morales & A. Fuentes | – | – | – | *Haemadictyon* |
| *P. brachypoda* S. F. Blake | – | *Tomentosae* | *Euprestonia* | * |
| *P. brittonii* N. E. Br. | – | *Annulares* | *Haemadictyon* | * |
| *P. calycina* Müll. Arg. | *Euprestonia* | – | – | *Prestonia* |
| *P. cayennense* (A. DC.) Pichon | – | – | *Haemadictyon* | *Haemadictyon* |
| *P. clandestina* J. F. Morales | – | – | – | *Prestonia* |
| *P. coalita* (Vell.) Woodson | – | *Coalitae* | *Rhaptocarpus* | *Coalitae* |
| *P. cogolloi* J. F. Morales | – | – | – | *Prestonia* |
| *P. concolor* (S. F. Blake) Woodson | – | *Annulares* | *Haemadictyon* | * |
| *P. cordifolia* Woodson | – | *Tomentosae* | *Euprestonia* | *Mollis* |
| *P. cyaniphylla* (Rusby) Woodson | – | *Acutifoliae* | *Haemadictyon* | *Haemadictyon* |
| *P. denticulata* (Vell.) Woodson | – | *Annulares* | *Haemadictyon* | *Denticulatae* |
| *P. dentigera* Woodson | – | – | *Haemadictyon* | * |
| *P. didyma* (Vell.) Woodson | – | *Annulares* | *Haemadictyon* | *Exsertae* |
| *P. discolor* Woodson | – | *Annulares* | *Haemadictyon* | * |
| *P. dusenii* (Malme) Woodson | – | *Coalitae* | *Rhaptocarpus* | *Coalitae* |
| *P. ecuadorensis* K. Schum. | *Haemadictyon* | * | *Haemadictyon* | * |
| *P. exserta* (A. DC.) Standl. | – | *Annulares* | *Haemadictyon* | *Exsertae* |
| *P. falcatosepala* J. F. Morales | – | – | – | *Exsertae* |
| *P. finitima* Woodson | – | *Annulares* | *Haemadictyon* | * |
| *P. folsomii* J. F. Morales | – | – | – | *Exsertae* |
| *P. gaudichaudii* (A. DC.) K. Schum. | *Haemadictyon* | * | *Haemadictyon* | * |
| *P. guatemalensis* Woodson | – | *Annulares* | *Haemadictyon* | * |
| *P. guianensis* Gleason | – | *Annulares* | *Haemadictyon* | * |
| *P. hammelii* J. F. Morales | – | – | – | *Prestonia* |
| *P. hassleri* Woodson | – | *Acutifoliae* | *Haemadictyon* | * |
| *P. haughtii* Woodson | – | – | *Haemadictyon* | *Denticulatae* |
| *P. hirsuta* Müll. Arg., nom. illeg. | *Euprestonia* | * | * | * |
| *P. ipomaeifolia* A. DC. | – | *Tomentosae* | *Euprestonia* | *Prestonia* |
| *P. isthmica* Woodson | – | *Tomentosae* | *Euprestonia* | * |
| *P. lacerata* Woodson | – | *Annulares* | *Haemadictyon* | - |
| *P. lagoensis* (Müll. Arg.) Woodson | – | *Acutifoliae* | *Haemadictyon* | *Haemadictyon* |
| *P. lanata* Müll. Arg. | *Euprestonia* | * | * | * |
| *P. latifolia* Benth. | *Euprestonia* | * | * | * |
| *P. laxa* Rusby ex Woodson | – | *Annulares* | *Haemadictyon* | * |
| *P. leco* A. Fuentes & J. F. Morales | – | – | – | *Denticulatae* |
| *P. lenticellata* A. H. Gentry | – | – | – | *Denticulatae* |
| *P. lindleyana* Woodson | – | *Annulares* | *Haemadictyon* | *Denticulatae* |
| *P. lindmanii* (Malme) Hoehne | – | *Acutifoliae* | *Haemadictyon* | * |
| *P. longifolia* (Sessé & Moc.) J. F. Morales | – | – | – | *Haemadictyon* |
| *P. longituba* K. Schum. | *Euprestonia* | * | * | * |
| *P. lutescens* Müll. Arg. | *Euprestonia* | * | * | * |
| *P. macroneura* Woodson | – | *Annulares* | *Haemadictyon* | *Denticulatae* |
| *P. macrophylla* Woodson | – | – | *Haemadictyon* | - |
| *P. marginata* Markgr. | – | *Acutifoliae* | *Haemadictyon* | *Haemadictyon* |
| *P. megagros* (Vell.) Woodson | – | *Annulares* | *Haemadictyon* | *Denticulatae* |

Table 2. Continued.

| | Schumann (1895) | Woodson (1936) | Pichon (1950a) | This study |
|---|---|---|---|---|
| *P. mexicana* A. DC. | *Euprestonia* | *Tomentosae* | *Euprestonia* | *Prestonia* |
| *P. mollis* Kunth | – | *Annulares* | *Haemadictyon* | *Mollis* |
| *P. mucronata* Rusby | – | *Tomentosae* | *Haemadictyon* | *Prestonia* |
| *P. obovata* Standl. | – | *Annulares* | *Haemadictyon* | * |
| *P. ornata* (Hoehne) J. F. Morales, M. E. Endress & Liede | – | – | – | *Coalitae* |
| *P. pachyphylla* Woodson | – | *Acutifoliae* | * | * |
| *P. papillosa* (Müll. Arg.) J. F. Morales | – | – | – | *Haemadictyon* |
| *P. parviflora* (Benth.) Benth. & Hook. f. | – | *Tomentosae* | *Tetraceras* | *Prestonia* |
| *P. parvifolia* K. Schum. ex Woodson | – | *Annulares* | *Haemadictyon* | *Exsertae* |
| *P. peregrina* Woodson | – | *Annulares* | *Haemadictyon* | * |
| *P. perplexa* Woodson | – | *Annulares* | *Haemadictyon* | * |
| *P. phenax* Woodson | – | *Annulares* | *Haemadictyon* | - |
| *P. pickelii* Markgr. | – | – | *Haemadictyon* | *Coalitae* |
| *P. plumierifolia* Markgr. | – | *Annulares* | *Haemadictyon* | *Denticulatae* |
| *P. portobellensis* (Beurl.) Woodson | – | *Annulares* | *Haemadictyon* | *Haemadictyon* |
| *P. premontana* J. F. Morales | – | – | – | *Prestonia* |
| *P. purpurissata* Woodson | – | *Annulares* | *Haemadictyon* | * |
| *P. quinquangularis* (Jacq.) Spreng. | – | *Acutifoliae* | *Haemadictyon* | *Haemadictyon* |
| *P. racemosa* J. F. Morales | – | – | – | *Prestonia* |
| *P. riedelii* (Müll. Arg.) Markgr. | – | *Tomentosae* | *Haemadictyon* | *** |
| *P. riverae* J. F. Morales | – | – | – | *Prestonia* |
| *P. robusta* Rusby | – | *Annulares* | *Haemadictyon* | *Denticulatae* |
| *P. rotundifolia* K. Schum. ex Woodson | – | *Annulares* | *Haemadictyon* | *Denticulatae* |
| *P. schippii* Woodson | – | *Annulares* | *Haemadictyon* | * |
| *P. schumanniana* Woodson | – | *Tomentosae* | *Euprestonia* | *Prestonia* |
| *P. seemannii* Miers | – | – | – | *Prestonia* |
| *P. simulans* Woodson | – | *Acutifoliae* | *Haemadictyon* | * |
| *P. solanifolia* (Müll. Arg.) Woodson | – | *Coalitae* | *Rhaptocarpus* | *Coalitae* |
| *P. speciosa* Donn. Sm. | – | *Tomentosae* | *Euprestonia* | *Prestonia* |
| *P. succo* J. F. Morales | – | – | – | ? |
| *P. surinamensis* Müll. Arg. | – | *Tomentosae* | *Euprestonia* | *Prestonia* |
| *P. tomentosa* R. Br. | – | *Tomentosae* | *Euprestonia* | *Prestonia* |
| *P. trifida* (Poepp.) Woodson | – | *Annulares* | *Haemadictyon* | *Denticulatae* |
| *P. tysonii* A. H. Gentry | – | – | – | *Prestonia* |
| *P. vallis* Woodson | – | *Annulares* | *Haemadictyon* | * |
| *P. vana* Woodson | – | *Annulares* | *Haemadictyon* | *Denticulatae* |
| *P. vaupesana* Woodson | – | – | – | *Haemadictyon* |
| *P. velutina* Woodson | – | *Annulares* | *Haemadictyon* | * |
| *P. versicolor* Woodson | – | *Annulares* | *Haemadictyon* | * |

*Treated as a synonym.
**Included in *Echites*.
***Included in *Rhodocalyx*.
Dash (–), not treated; question mark (?), incertae sedis.

species and the second from a morphotype that matches the type of *P. cordifolia* (cordate and pubescent leaves and purple corollas) and one that matches the type of *P. marginata* (coriaceous leaves from the Guiana Shield). The list of taxa, vouchers, and GenBank accession numbers are given in Appendix 1. Eight species of *Prestonia* (*P. amazonica* (Benth. ex Müll. Arg.) J. F. Macbr., *P. cogolloi* J. F. Morales, *P. folsomii* J. F. Morales, *P. haughtii* Woodson, *P. macroneura* Woodson, *P. megagros* (Vell.) Woodson, *P. racemosa* J. F. Morales, and *P. succo* J. F. Morales) were not included in this study. These species are poorly collected and few specimens for DNA extraction are available. We tried to obtain positive polymerase chain reaction (PCR) products from several specimens without success.

### DNA extraction, amplification, and sequencing

DNA extraction, amplification, primers, and sequencing procedures for the *matK* + *5′/3′ trnK* intron, *rpl16* intron,

*rps16* intron, *trnL* intron + *trnL-trnF* intergenic spacer, and ITS are described in Morales et al. (2017). For the *rps16* intron, the protocol given in Simões et al. (2004) was used. The primer 825R (5′-GATAGCATAGTGCGATAGAGTC-3′) was designed for use in sequencing the *5′ trnK* intron in combination with the primer trnK-3914F. For sequences already available in GenBank, sometimes a different voucher specimen was used to obtain ITS. Alignment of the *matK* + *5′/3′ trnK* intron, *trnL* intron + *trnL-trnF* intergenic spacer, and *rps16* intron was relatively easy and straightforward, whereas alignment of ITS was the most difficult due to the large number of gaps, followed by the *rpl16* intron, which presented large mononucleotide repeats. Regions of ambiguous alignments were excluded. Sequences were manually assembled using CodonCode Aligner version 3.7.1 (CodonCode Corp., Centerville, Massachusetts, U.S.A.).

### PHYLOGENETIC ANALYSES

We analyzed seven matrices: the individual datasets (1) *matK* + *5′/3′ trnK* intron; (2) *rpl16* intron; (3) *rps16* intron; (4) *trnL* intron + *trnL-trnF* intergenic spacer; (5) ITS; (6) combined cpDNA dataset (matrices 1–4); and (7) combined molecular dataset (matrices 5–6). All matrices were deposited in TreeBASE (accession number 19980). First we checked for hard incongruence among the individual plastid matrices, and then between the combined cpDNA matrix and the nrDNA matrix, testing the combinability of the partitions by searching for incongruences between them, in order to combine the data following the procedures given in Morales et al. (2017). The individual cpDNA matrices were analyzed using maximum likelihood (ML), whereas the combined cpDNA matrix, the nrDNA matrix, and the combined molecular matrix were analyzed using ML and Bayesian inference (BI). For the two analyses involving ITS, the nrDNA matrix was divided into five partitions.

Maximum parsimony (MP) analyses were performed in PAUP* 4b10 (Swofford, 2002), with characters equally weighted and unordered. Heuristic searches were performed with tree bisection and reconnection (TBR) branch swapping on 1000 random addition sequences, holding up to 10 trees per search. The most parsimonious trees were swapped to completion. Tree length, consistency index (CI), retention index (RI), and bootstrap support (BS) were calculated based on informative characters. Relative support for each node was estimated using the bootstrap resampling procedure (Felsenstein, 1985) as implemented in PAUP, employing a heuristic search with 1000 pseudo-replicates followed by TBR swapping. Each replicate was performed with a single random addition sequence and holding up to 20 trees. The strict consensus of each replicate was saved to calculate the bootstrap, and congruence was assessed by nodes with greater than 50% BS. BS value was interpreted as follows: 50%–74% as weak, 76%–84% as moderate, and ≥ 85% as strongly supported (Simões et al., 2016).

ML analyses were calculated using RAxML version 8.2.8 (Stamatakis, 2006; Stamatakis et al., 2008), as implemented in CIPRES version 3.3 (Miller et al., 2010), setting the non-bootstrap analysis and tree search to 1000 replicates. The output tree files were generated with Figtree (Rambaut, 2014). BS value was interpreted as follows: 50%–74% as weak, 75%–89% as moderate, and ≥ 90% as strongly supported.

BI was calculated using MrBayes version 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), applying separate models to each data partition, with unlinked partitions, and parameters estimated independently. Models of sequence evolution were selected using the Bayesian information criterion (BIC) in jModelTest version 2.1.1 (Darriba et al., 2012). MrBayes was run using two parallel runs for 10 million generations, each using one cold and three heated Markov chain Monte Carlo (MCMC) chains, sampling every 10,000 generations, until an average standard deviation of split frequencies ≤ 0.01 was reached. The first 25% of the trees was discarded (sump function) and the remaining 75% used to calculate a majority-rule consensus and posterior probabilities (PP, sumt function). Burn-in values, mixing of the MCMC chains, and independent tree sampling were determined through inspection of the MCMC samples using Tracer v.1.5 (Rambaut & Drummond, 2007). The 50% majority-rule consensus tree was edited in Figtree version 1.4.2. (Rambaut, 2014). Posterior probability values ≥ 0.95 were considered as strongly supported.

## RESULTS

### SEQUENCES

The markers chosen for this investigation were successfully sequenced for all species, with the following exceptions. For the outgroup species *Asketanthera calycosa* (A. Rich.) Woodson, we were unable to amplify the *rps16* intron. For *Prestonia mollis* and *P. cordifolia*, despite several amplifications from two different specimens, ITS sequences always exhibited double overlapping peaks, a problem already reported with this marker (Rapini et al., 2006; Spalik et al., 2009; Burge et al., 2013; Weitemier et al., 2015; Simões et al., 2016). Therefore, for these three species ITS was not included, which was not expected to affect the results of the combined analysis (Wiens, 2003). In total, 62 nrDNA and 258 cpDNA sequences were analyzed, of which 46 and 205, respectively, were newly generated; the remaining sequences were obtained from GenBank. Species sampled, vouchers, and GenBank accession numbers are given in Appendix 1. Alignment characteristics statistics are summarized in Table 3. The most informative of the

Table 3. Summary statistics of data sets.

| Locus | Number of taxa | Total length (bp) | Variant characters (n) | Parsimony-informative characters (n) | CI | RI |
|---|---|---|---|---|---|---|
| *matK* + *5′/3′ trnK* intron | 65 | 2585 | 395 | 224 (8.66%) | 0.623 | 0.841 |
| *rpl16* intron | 65 | 1229 | 210 | 111 (9.03%) | 0.734 | 0.890 |
| *rps16* intron | 63 | 956 | 149 | 96 (10.04%) | 0.710 | 0.889 |
| *trnL* intron + *trnL-trnF* intergenic spacer | 65 | 991 | 109 | 65 (6.6%) | 0.780 | 0.912 |
| ITS | 62 | 812 | 490 | 383 (47.17%) | 0.536 | 0.763 |
| Chloroplast combined | 65 | 5761 | 863 | 496 (8.61%) | 0.645 | 0.846 |
| ITS and chloroplast combined | 65 | 6573 | 1353 | 879 (13.37%) | 0.574 | 0.795 |

CI, consistency index; RI, retention index.

regions studied was ITS, with 47.17% parsimony informative characters (Table 3). In contrast, the most informative cpDNA markers (*rps16* intron, *rpl16* intron) barely reached 10% (10.04% and 9.03%, respectively).

### DNA analyses

The most complex model GTR + $\Gamma$ + I was implemented for ITS, model TVM+g was selected for *matK*, and model GTR + g was selected for *rpl16* intron, *rps16* intron, *trnL* intron + *trnL-trnF* intergenic spacer, and *5′/3′ trnK* intron. No strongly supported incongruent clades were found between the *rpl16* intron (Fig. S1; for Figs. S1–S4, see https://www.researchgate.net/publication/320024812_Systematics_of_Prestonia_Apocynaceae_Apocynoids_Echiteae_Eighty_years_after_Woodson), *rps16* intron (Fig. S2), and *trnL* intron + *trnL-trnF* intergenic spacer (Fig. S3) trees. In the *matK* + *5′/3′ trnK* intron tree (Fig. S4), *Artia* and *Parsonsia* were retrieved in an unsupported clade with a group of species of *Prestonia*. In the combined cpDNA tree (Fig. 1) and the nrDNA tree (Fig. 2), no well-supported conflicts were found (maximum parsimony jackknife [MPJK] or maximum parsimony bootstrap [MPBS] > 70%; Mason-Gamer & Kellogg, 1996; Zhang & Simmons, 2006). In the combined cpDNA tree, the maximally supported (posterior probability [PP] = 1, maximum likelihood bootstrap [MLBS] = 100%, MPBS = 100%) *Mollis* clade (comprising *P. mollis* and *P. cordifolia*) was retrieved as sister to the *Coalita*, *Denticulata*, and *Prestonia* clades, whereas in the nrDNA tree *P. mollis* and *P. cordifolia* were not included and, therefore, the *Mollis* clade is missing. Therefore, the two datasets were combined into a single matrix (combined molecular matrix). All further discussion is based on the majority rule consensus cladogram resulting from the BI analysis of the combined molecular matrix (Fig. 3).

### phylogenetic relationships

*Prestonia* as circumscribed by Morales et al. (2017) is optimally resolved as monophyletic (1/95/64). Five main clades can be recognized: the *Haemadictyon* clade, *Mollis* clade, *Coalita* clade, *Denticulata* clade, and *Prestonia* clade (Fig. 3). The first clade to diverge within the ingroup, the *Haemadictyon* clade is strongly supported by PP (1), but only moderately so by ML (BS = 80%) and unsupported by MP. It is retrieved as sister to all other clades in *Prestonia* and is divided into two clades. The first clade (1/100/100) includes two species, *P. marginata* and *P. quinquangularis*, and the second (0.95/67/78), nine species; it is further divided into two clades, both optimally supported (1/100/100), comprising three and six species, respectively. Next in branching order is the strongly supported *Mollis* clade (1/100/100), which comprises only two species (*P. cordifolia* and *P. mollis*). The relationship between the *Mollis* clade and the *Haemadictyon* clade is strongly supported by PP (0.99), but only weakly so by ML (BS = 72) and MP (BS = 51). This clade is resolved as sister (1/100/75) to the large clade comprising the *Coalita*, *Denticulata*, and *Prestonia* clades, of which the *Coalita* clade branches off first. It is strongly supported (1/98/91) and comprises two clades: one strongly supported (1/98/96) with four species, and the second optimally supported (1/100/100) with five species. Next in branching sequence is the large clade (1/100/100), which includes the bulk of the species in the genus and is divided into two clades: the *Denticulata* clade (0.95/83/88) and the *Prestonia* clade (1/100/99), with 11 and 17 species, respectively.

## Discussion

The present study presents the first densely sampled phylogeny of the Neotropical lianoid genus *Prestonia*, using combined cpDNA (*matK* + *5′/3′ trnK* intron, *rpl16* intron, *rps16* intron, and *trnL* intron + *trnL-trnF* intergenic spacer) and nrDNA (ITS). The inclusion of nrDNA in this study provided a significantly increased phylogenetic signal, compared to the results based only on cpDNA markers. Similar results have been found in

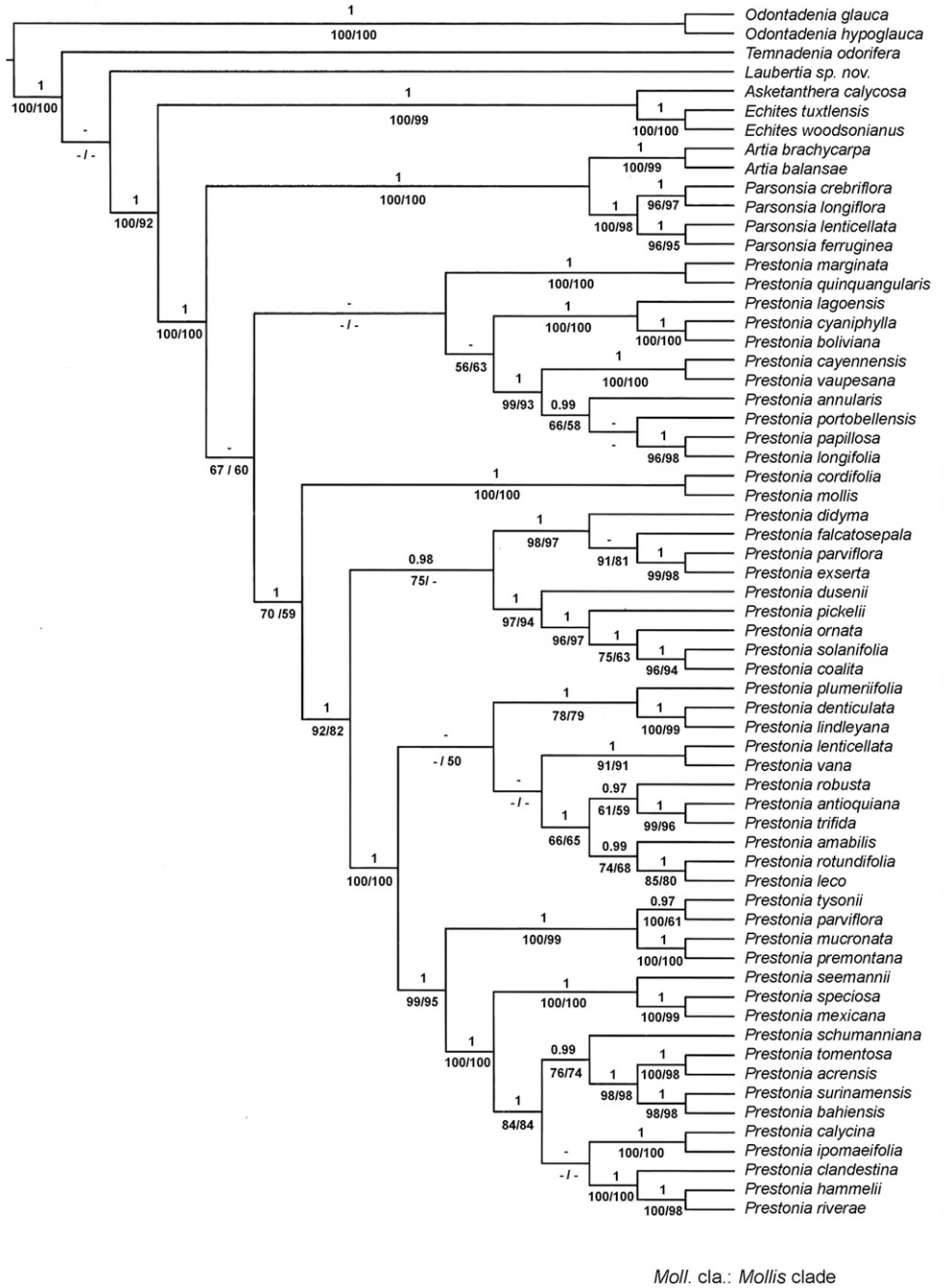


Figure 1. Maximum likelihood consensus tree based on the combined cpDNA dataset (*matK* + *5′/3′* intron, *trnK* intron, *rpl16* intron, *rps16* intron, and *trnL* intron + *trnL-trnF* intergenic spacer). Clades from the consensus tree are based on the combined molecular dataset (combined cpDNA and nrDNA datasets) (Fig. 3) are indicated by black bars. Bayesian posterior probabilities ($\geq 0.95$) are indicated above each branch, and maximum likelihood bootstrap support and maximum parsimony bootstrap support ($> 50\%$) are indicated below.

other studies in Neotropical apocynoids (e.g., Livshultz, 2010; Morales et al., 2017). The ITS dataset proved to be the most informative, with ca. 47% of the aligned characters being parsimony informative. The enhanced variability of nuclear loci compared to other markers provides great potential in phylogenetic studies. However,

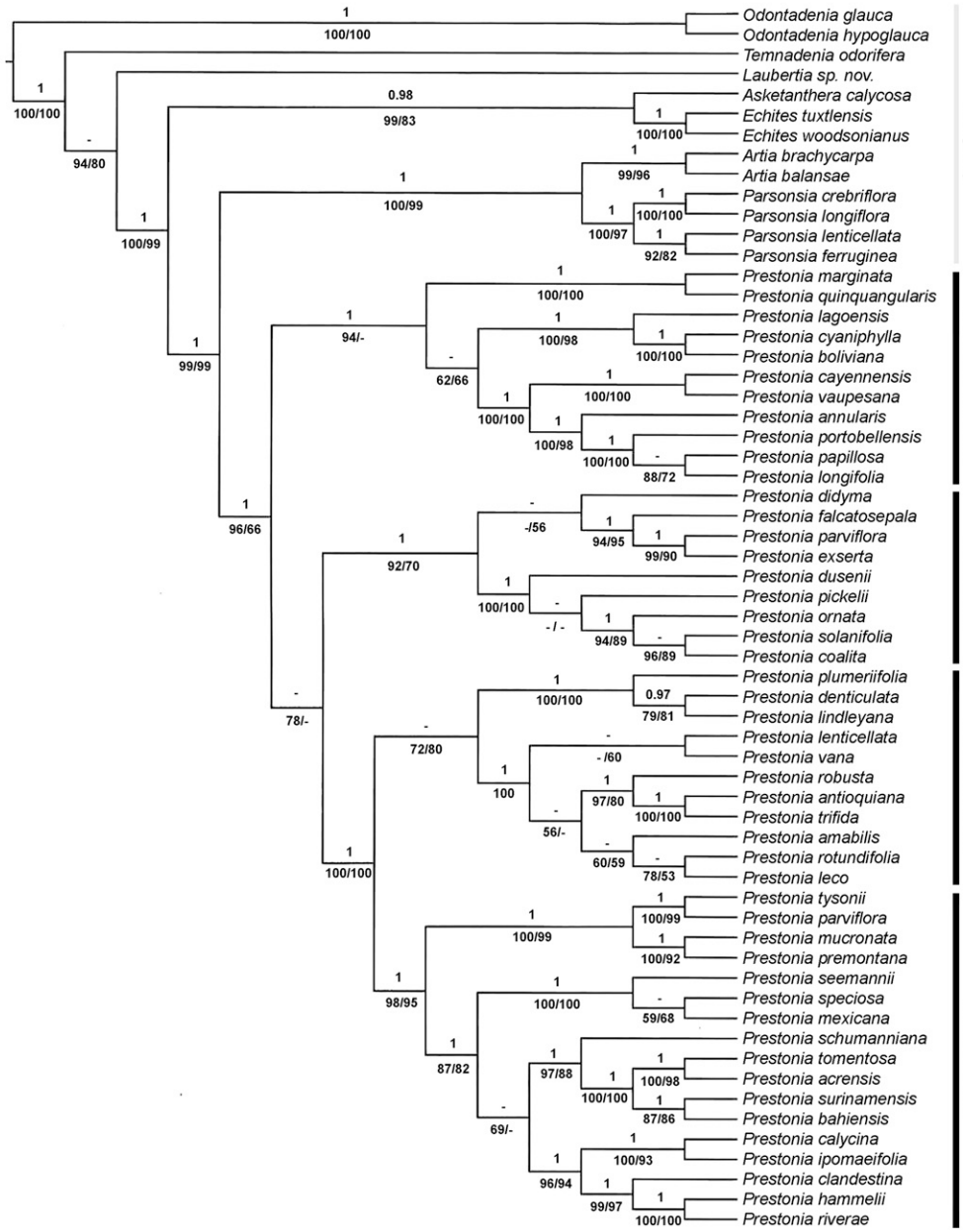


Figure 2. Maximum likelihood consensus tree based on the nrDNA (ITS) dataset. Clades from the consensus tree based on the combined molecular dataset (Fig. 3) are indicated by black bars. Bayesian posterior probabilities (≥ 0.95) are indicated above each branch, and maximum likelihood bootstrap support and maximum parsimony bootstrap support (> 50%) are indicated below.

the design and identification of universal primers are somewhat difficult due to the polyploidy present in plants (Naumann et al., 2011). Here, the nrDNA data are congruent with those based on cpDNA data; however, incongruence between nuclear and plastid data is not uncommon (e.g., Fan et al., 2009; Pelser et al., 2010; Silva et al., 2012).

### PHYLOGENY COMPARED TO PREVIOUS CLASSIFICATIONS

The broader taxon sampling allowed us to corroborate the hypothesis of monophyly for the genus after the taxonomic changes proposed by Morales et al. (2017), and to evaluate the sections of three previous infrageneric classification systems: Schumann (1895), Woodson (1936), and Pichon (1950a). Almost all sections

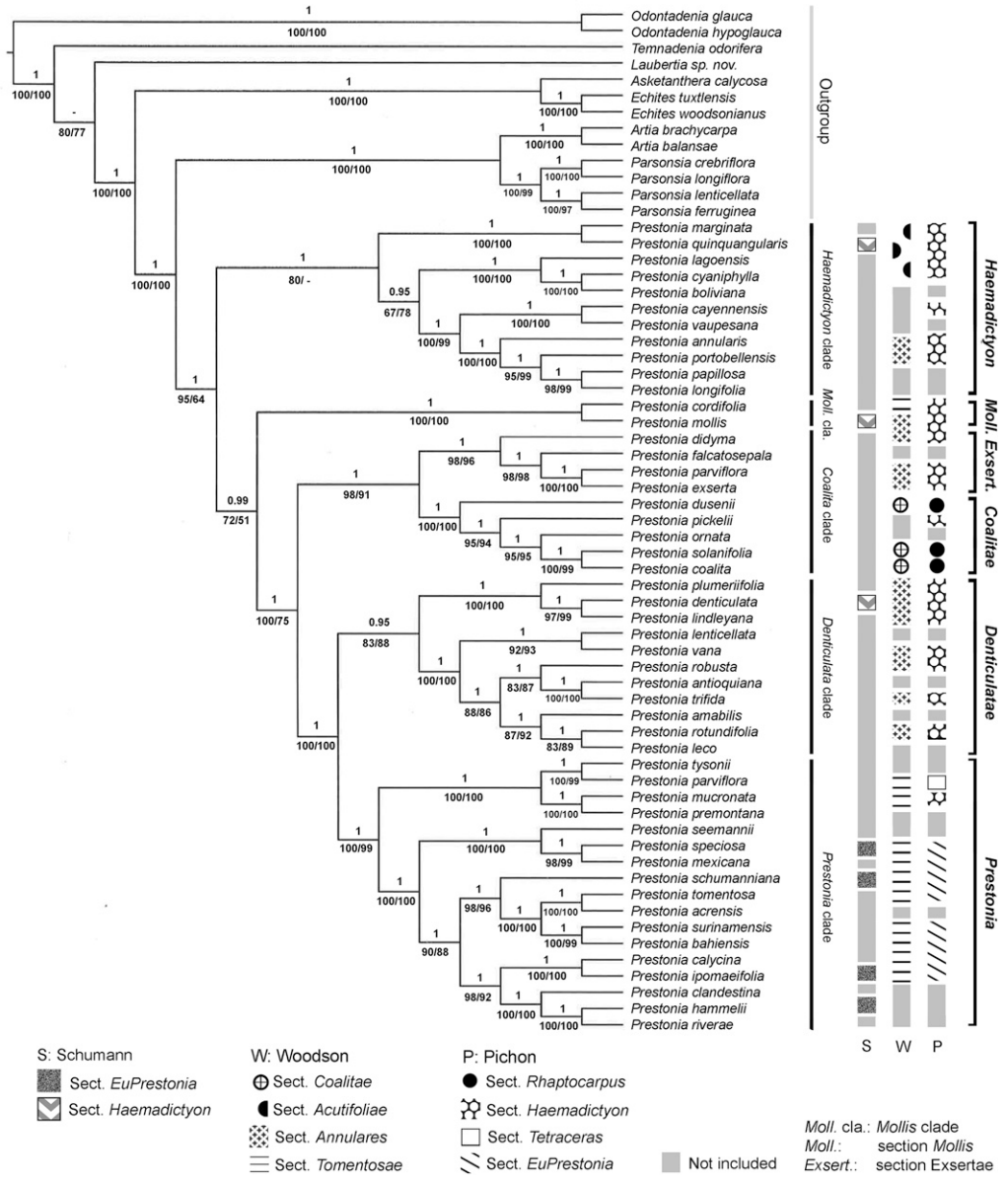


Figure 3. Maximum likelihood consensus tree based on the combined molecular dataset. Clades are indicated by thin black bars in the innermost column. Sections in the classifications of Schumann (1895), Woodson (1936), and Pichon (1950a) are shown by patterns in the three middle columns labeled as S, W, and P, respectively. The new sectional classification proposed here is indicated by thin black bars in the outermost column with section names in bold. Bayesian posterior probabilities ($\geq 0.95$) are indicated above each branch, and maximum likelihood bootstrap support and maximum parsimony bootstrap support ($> 50\%$) are indicated below.

of these are para- or polyphyletic. Schumann's *Prestonia* sect. *Euprestonia* is resolved as monophyletic. In Woodson's and Pichon's systems, only *Prestonia* sect. *Coalitae* and *Prestonia* sect. *Rhaptocarpus* are monophyletic, respectively. The most extreme example of polyphyly is found in Schumann's *Prestonia* sect. *Haemadictyon*, Woodson's *Prestonia* sect. *Annulares*, and Pichon's *Prestonia* sect. *Haemadictyon*, the constituent species of which are scattered among three, five, and six clades, respectively. In Woodson's classification, *Prestonia* sect. *Acutifoliae* is also polyphyletic, with species in two different clades, and *Prestonia* sect. *Tomentosae* is paraphyletic, because here *P. cordifolia* is grouped in the *Mollis* clade. In Pichon's classification, the sole species of *Prestonia* sect. *Tetraceras* Pichon, *P. parviflora* (Benth.) Benth. & Hook. f., is nested among species of *Prestonia*

sect. *Euprestonia*, and *Prestonia* sect. *Rhaptocarpus* is polyphyletic, with its members grouped with two species of *Prestonia* sect. *Haemadictyon*.

Below, we discuss each of the five main clades recovered in *Prestonia* and their constituent species compared to the three previous infrageneric classifications by Schumann (1895), Woodson (1936), and Pichon (1950a). For the remainder of the discussion, we refrain from citing these three references. Many of the species included by Schumann are today treated as synonyms; therefore, in Figure 3 we used the currently accepted names, but the complete list is given in Table 2.

### *HAEMADICTYON* CLADE

Of the 11 species in this clade, four were described or accepted after 1950 (*Prestonia boliviana* J. F. Morales & A. Fuentes, *P. longifolia* (Sessé & Moc.) J. F. Morales, *P. papillosa* (Müll. Arg.) J. F. Morales, and *P. vaupesana* Woodson) and thus not treated by Schumann, Woodson, or Pichon. Of the remaining seven, four (*P. cyaniphylla* (Rusby) Woodson, *P. lagoensis* (Müll. Arg.) Woodson, *P. marginata*, and *P. quinquangularis* (as *P. acutifolia* (Benth. ex Müll. Arg.) K. Schum.)) were included by Woodson in *Prestonia* sect. *Acutifoliae* and another two (*P. annularis* (L. f.) G. Don and *P. portobellensis* (Beurl.) Woodson) in *Prestonia* sect. *Annulares*. The basionym of *P. cayennense* (A. DC.) Pichon, *Haemadictyon cayennense* A. DC., was not treated in Woodson's monograph. Schumann's *Prestonia* sect. *Haemadictyon* is polyphyletic, since its three species were retrieved in three different clades (*Haemadictyon*, *Mollis*, and *Denticulata*). Woodson's *Prestonia* sect. *Acutifoliae* originally included nine species, five of which have been reduced to synonymy in recent years (Morales, 2004b, 2005a, 2007b); it is monophyletic, but was retrieved in a close relationship with a group of species included by him in *Prestonia* sect. *Annulares*. Fifteen of the 33 species accepted by Woodson in his original circumscription of *Prestonia* sect. *Annulares* (Table 1) have been reduced to synonymy and its remaining representatives are divided among three clades, confirming that section as polyphyletic as well.

*Prestonia* sect. *Haemadictyon* as defined by Pichon was resolved as grossly polyphyletic here, with its members distributed within all major clades. We propose to recognize the *Haemadictyon* clade as a section. Since the type of *Prestonia* sect. *Haemadictyon* is included here, Baillon's section name is maintained, but with a different species circumscription.

Species from this clade are distributed from Mexico and some Caribbean Islands to Bolivia and northern Argentina from 0 to 1600 m, but are mostly confined to South America, with only three species (*Prestonia longifolia*, *P. portobellensis*, and *P. quinquangularis*) reaching Central America and Mexico. They have in common sepals without longitudinal veins, a glabrous corolla with an evident annular corona, free corona lobes with the apices exserted or included, and follicles that are free or that remain longitudinally fused together up to maturity. The species are grouped into three clades. The five species of the first and second clade are characterized by membranaceous, glabrous to minutely puberulent leaf blades and membranaceous sepals and are found in cerrados, dry forest, gallery forest, and open areas from southern Nicaragua and Caribbean Islands to northern Argentina. The six species of the third clade have subcoriaceous, glabrous leaf blades and firmly membranaceous sepals and typically grow in wet forest or tropical wet forest from Mexico to Bolivia and northern Brazil.

*Prestonia quinquangularis* is one of the most variable species in the genus regarding shape, texture, and indument of the leaves. Specimens from dry forest have small, completely glabrous leaves, whereas specimens from the tropical wet forest from the Amazonian basin and the Guianas tend to have larger leaves, which may be sparsely puberulent. The relationships of *P. marginata* have been disputed in some more recent floras and checklists. For example, Zarucchi et al. (1995), Funk et al. (2007), and Hokche et al. (2008) all included this species in the synonymy of *P. cayennense*, whereas Morales (2007b) considered it to be a synonym of *P. quinquangularis*. We included a specimen of *P. marginata* here in order to determine its taxonomic affinities. It is closely related to *P. quinquangularis*, supporting the proposal of Morales (2007b). The status of *P. marginata* will be addressed in the upcoming monograph of *Prestonia* (Morales & Endress, unpubl. data). Pichon's *Prestonia* sect. *Haemadictyon* (based on Schumann's *Prestonia* sect. *Haemadictyon*) and Woodson's *Prestonia* sect. *Acutifoliae* have the same type (*Prestonia acutifolia* [now = *P. quinquangularis*]), rendering Woodson's name a nom. superfl. Baillon (1891) proposed *Prestonia* sect. *Haemadictyon*, based on *Haemadictyon* Lindl., with *H. venosum* Lindl. as the type. Although Baillon did not cite types for his sections, it was not until 1958 that the citation of a type became compulsory (Art. 40.1 of the International Code of Nomenclature; McNeill et al., 2012). Up until 1908 (Art 38.7) only a brief description, a reference to a description previously published (Art. 38.1), or an illustration with analysis was sufficient for a name to be effectively published. Here, we maintain Baillon's name for *Prestonia* sect. *Haemadictyon*, but with a different species composition.

### *MOLLIS* CLADE

Schumann included *Prestonia ecuadorensis* K. Schum. (reduced to the synonymy of *P. mollis* by

Woodson [1936]) in his *Prestonia* sect. *Haemadictyon*. In Woodson's key to *Prestonia*, *P. mollis* came out in the same group with *P. exserta* (A. DC.) Standl. (including *P. tobagensis* Urb. and *P. velutina* Woodson [Morales 2004b, 2006]), *P. parvifolia* K. Schum. ex Woodson, and *P. didyma* (Vell.) Woodson (including *P. perplexa* Woodson [Morales, 2007b]). However, *P. exserta*, *P. parvifolia*, and *P. didyma* are nested in the *Coalita* clade. Woodson included *P. cordifolia* in *Prestonia* sect. *Tomentosae*, based on its tomentulose leaves and puberulent corollas, and hypothesized that its closest relative was *P. calycina* Müll. Arg. (Woodson, 1936: 354), a species here included in the distant *Prestonia* clade. Pichon included both species of the *Mollis* clade in *Prestonia* sect. *Haemadictyon* (which included 50 species in total). Although we were not able to include nrDNA data in our analysis for *P. mollis* and *P. cordifolia*, we propose to recognize this clade as a section, based on its high support (1/100).

Species of the *Mollis* clade are distributed in Ecuador and northern Peru, with a disjunct population in Central Panama, where they are found mainly in disturbed areas or dry forest from sea level up to 2500 m. They are characterized by membranaceous, glabrous to minutely puberulent leaf blades, membranaceous sepals with evident longitudinal veins, glabrous or minutely puberulent corollas, well-developed annular corona, free corona lobes with the apices exserted, and follicles that remain longitudinally fused together up to maturity. At higher elevations, the plants tend to be more pubescent, with a uniform indument on stems, leaves, and inflorescences. Corolla color ranges from purple or yellowish purple to yellow. Plants from high elevations were described by Woodson as *Prestonia cordifolia*, which is possibly an extreme morphotype of the variable *P. mollis*, with purple corollas and larger leaves. Following Woodson (1936), *P. cordifolia* has been accepted in some floristic works focusing on Peru (e.g., Macbride, 1959; León et al., 2006), without questioning its validity. Whether or not both species should be accepted is a question that will be addressed in the upcoming monograph of *Prestonia*.

### *COALITA* CLADE

The strongly supported *Coalita* clade (1/95) is divided into two clades. The first (0.99/65) comprises four species: *Prestonia didyma*, *P. parvifolia*, *P. exserta*, and *P. falcatosepala* J. F. Morales. The last was described only in 2004, and, consequently, was not included in previous infrageneric proposals. The first three species were considered by Woodson to be related to *P. annularis* and *P. mollis* (which are placed in different, distant clades here) and thus he included them as part of *Prestonia* sect. *Annulares*, whereas they were included in *Prestonia* sect. *Haemadictyon* in Pichon's classification. The second clade (1/100) includes all species of Woodson's *Prestonia* sect. *Coalitae* as well as Pichon's *Prestonia* sect. *Rhaptocarpus*. None of the species of the *Coalita* clade were treated by Schumann, because some were not yet described or were placed under a different genus (e.g., *Echites*).

Although the first clade is only weakly supported by ML, it is strongly supported by PP (1). Furthermore, species of the first clade are characterized by a well-developed annular corona and free corona lobes versus inconspicuous annular corona and free corona lobes absent in the second clade. Therefore, we propose to recognize the first clade as a new section, *Prestonia* sect. *Exsertae* J. F. Morales, M. E. Endress & Liede.

Representatives of this clade grow in dry, moist, or tropical wet forest and bordering zones in Central Panama, Colombia, Ecuador, Peru, and southeastern Brazil, from 0 to 1100 m. All species have membranaceous leaf blades that are glabrous (or sparsely puberulent on the abaxial surface), membranaceous sepals, glabrous corollas, well-developed annular corona, free corona lobes with the apices exserted (sometimes almost totally exserted), and follicles that are free or that remain longitudinally fused together up to maturity.

Two of the included species in the second clade, *Prestonia ornata* (Hoehne) J. F. Morales, M. E. Endress & Liede and *P. pickelii* Markgr., have a complex taxonomic history. *Prestonia ornata* was originally described in *Echites* (Hoehne, 1915) and was transferred to *Temnadenia* by Woodson (1932b), which was accepted by Morales (2005b). However, in a recent molecular-based study of the tribe Echiteae, the included sample of *T. ornata* grouped within *Prestonia*, thus the formal transfer was made (Morales et al., 2017), an action that is supported with the larger sampling here. *Prestonia pickelii* was described by Markgraf in 1938, too late to be treated in Woodson's monograph; Pichon placed it in *Prestonia* sect. *Haemadictyon*, thus causing his *Prestonia* sect. *Rhaptocarpus* to be paraphyletic. *Prestonia pickelii* is known from only two collections from Pernambuco State, Brazil. It was reduced to the synonymy of *P. quinquangularis* (Morales, 2007b), based on certain morphological similarities (e.g., leaf shape, inflorescence type, small flowers, scarcely evident annular corona). According to our phylogeny, however, the two are not even closely related; therefore, *P. pickelii* is reinstated here as a valid species. We propose to recognize this second clade as a section, with Woodson's name *Prestonia* sect. *Coalitae* maintained, but with a different species circumscription.

Species of this clade are typically found in dry forest and cerrados, in Colombia to Bolivia and Paraguay from 0 to 1000 m, although *Prestonia ornata* grows in tropical wet forest. Representatives of this clade are characterized

by membranaceous leaf blades that are glabrous (or sparsely puberulent on the abaxial surface), membranaceous sepals, longitudinal veins absent or present, glabrous or minutely puberulent corollas, inconspicuous annular corona, free corona lobes usually absent, and follicles that are free or that remain longitudinally fused together up to maturity. Species without a free corona are uncommon and, outside of the species in this clade, have been reported in only a few species of the *Prestonia* clade.

### *DENTICULATA* CLADE

Of the 15 currently recognized species in this clade, seven were treated in Woodson's *Prestonia* sect. *Annulares* or Pichon's *Prestonia* sect. *Haemadictyon*, whereas Schumann included only *P. denticulata* (Vell.) Woodson (as *P. gaudichaudii* A. DC.) in his *Prestonia* sect. *Haemadictyon*. The other four species (*P. amabilis* J. F. Morales, *P. antioquiana* J. F. Morales & Liede, *P. lenticellata* A. H. Gentry, and *P. leco* A. Fuentes & J. F. Morales) were all described after 1950, and thus were not treated in any previous classification. Three species resolved here in the *Denticulata* clade (*P. robusta* Rusby, *P. rotundifolia* K. Schum. ex Woodson, and *P. trifida* (Poepp.) Woodson) were included in the study by Morales et al. (2017), where they also formed a strongly supported clade. Other phylogenetic studies dealing with relationships in the apocynoids (Livshultz et al., 2007; Livshultz, 2010) did not include any species of this clade. The clade is highly supported by PP (0.99) (ML-BS = 85/MP-BS = 88); accordingly, it is proposed as a new section, *Prestonia* sect. *Denticulatae* J. F. Morales, M. E. Endress & Liede.

Members of this clade are restricted to tropical wet forest regions from southern Costa Rica to Bolivia and Brazil, from 0 to 1000 m. Several of them are poorly collected because they grow in isolated areas of the Amazonian basin. The plants tend to be robust, woody lianas. They have coriaceous, glabrous leaf blades, glabrous or minutely puberulent corollas, evident annular corona, free corona lobes with the apices exserted or included, and follicles that are free or that remain longitudinally fused together up to maturity.

### *PRESTONIA* CLADE

The *Prestonia* clade is divided into two main clades. The smaller of the two includes four species, all of them characterized by terminal inflorescences, small corollas, and elongated linear, free follicles, whereas the larger comprises 13 species, which have axillary inflorescences, longer corollas, and stouter follicles.

All species of the *Prestonia* clade that were known at the time were included by Schumann in *Prestonia* sect. *Euprestonia* and by Woodson in *Prestonia* sect. *Tomentosae*, whereas they were placed in three different sections in Pichon's classification. The smaller clade includes *P. mucronata* Rusby from Pichon's *Prestonia* sect. *Haemadictyon* and *P. parviflora* (Benth.) Benth. & Hook. f., the sole species of his *Prestonia* sect. *Tetraceras*, which he separated from his *Prestonia* sect. *Euprestonia* based on the short corolla tube, ovules 6-seriate, and linear follicles (vs. longer corolla tube, ovules 8- to 12-seriate, and stouter follicles). Although *P. mucronata* has the same diagnostic features as *P. parviflora*, because its fruits were unknown at the time, Pichon erroneously placed it in *Prestonia* sect. *Haemadictyon*; thus, both sections are paraphyletic. All the species in the larger clade that were known at the time were included by Pichon in his *Prestonia* sect. *Euprestonia*. We propose to recognize the *Prestonia* clade as a section, *Prestonia* sect. *Prestonia*.

Species of the *Prestonia* clade are found from Mexico to Bolivia and northern Argentina, growing in a wide range of environments, including forest and open areas from sea level to 2500 m. Species of *Prestonia* from montane or premontane forest are found only in this clade. The species are characterized by membranaceous leaves with pubescent blades, membranaceous sepals, conspicuously pubescent corollas, evident annular corona, sometimes deeply lobed or reduced to five conical projections, free corona lobes with the apices exserted or included or absent, and free follicles.

### PLACEMENT OF SPECIES NOT SAMPLED

Based on their morphological features, we suggest possible placement of the eight species not sampled in the proposed infrageneric classification as follows: *Prestonia amazonica*, *P. haughtii*, *P. macroneura*, and *P. megagros* are placed in *Prestonia* sect. *Denticulatae* based on the coriaceous and glabrous leaf blades, coriaceous sepals without visible veins, corolla with conspicuous annular corona and free corona lobes, and follicles somewhat woody at maturity; *P. cogolloi* and *P. racemosa* are placed in *Prestonia* sect. *Prestonia* based on the corolla tube, abaxial surfaces of the corolla lobes, and follicles variously pubescent, with the indument ferrugineous or brown; and *P. folsomii* is placed in *Prestonia* sect. *Exsertae* based on membranaceous leaf blades and sepals, sepals with conspicuous longitudinal veins, and corolla tube 11–14 mm long, with a well-developed annular corona and free corona lobes. The affinities of *P. succo* cannot be determined at this time, because its features (e.g., small membranaceous leaves, small corollas, a scarcely evident corona, and absence of free corona lobes) do not match any other known species. Based on its vegetative characters it resembles the members of *Prestonia* sect. *Coalitae*, but without molecular evidence no further discussion is possible.

## Taxonomic Implications

**Prestonia** R. Br., Asclepiadeae, 58. 1810, nom. cons. TYPE: *Prestonia tomentosa* R. Br. (preprint of Mem. Wern. Nat. Hist. Soc. 1: 70. 1811).

Lianas or vines; bark conspicuously suberose; latex white or translucent; stems with intrapetiolar colleters. Leaves opposite, blades without colleters adaxially, glabrous or variously pubescent, membranaceous to coriaceous or subcoriaceous, sometimes revolute at the margin. Inflorescence a monochasial or dichasial cyme, axillary, terminal or subterminal, few- to many-flowered, glabrous to variously pubescent, bracts scarious to foliaceous. Sepals 5, free, diminutive to foliaceous, with 1 episepalous colleter at the base inside. Corolla salverform, rarely infundibuliform, glabrous to variously pubescent, lobes dextrorsely contort; annular corona usually present around the mouth, entire to variously lobed, 5 free corona lobes usually within the staminal sectors of the tube, with the apices exserted or deeply included, sometimes free corona lobes absent. Stamens included or with the apices exserted, anthers connivent and agglutinated to the style-head, filaments short, densely hirsute. Style-head spool-shaped; ovules many, several-seriate; nectary annular, variously lobed, sometimes divided into 5 individual nectaries. Follicles 2, apocarpous, usually free but united just at their apices, sometimes completely longitudinally united through maturity, rarely winged, glabrous or variously pubescent. Seeds numerous, dry, truncate, glabrous, comose at the micropylar end. Ca. 58 species.

The key to the sections in the new classification is based on the morphological descriptions in the new monograph for the genus (Morales & Endress, unpubl. data).

### Key to the Sections of *Prestonia*

1. Intrapetiolar colleters usually more than 1.5 mm long, well developed; corolla tube, abaxial surfaces of the corolla lobes, and follicles variously pubescent, the indument ferrugineous, brown, or yellow . . . . . *Prestonia* R. Br. sect. *Prestonia*
1′. Intrapetiolar colleters less than 1 mm long, very small; corolla tube, abaxial surfaces of the lobes, and follicles glabrous, glabrescent, or sparsely puberulent, the indument green or colorless . . . . . 2
2. Leaf blades and sepals coriaceous to subcoriaceous; follicles somewhat woody . . . *Prestonia* sect. *Denticulatae* J. F. Morales, M. E. Endress & Liede
2′. Leaf blades, sepals, and follicles membranaceous . . . . . 3
3. Annular corona scarecely evident, less than 0.4 mm long, sometimes absent; corolla tube usually without free corona lobes within (except *P. pickelii* Markgr.) . . . . . *Prestonia* sect. *Coalitae* Woodson
3′. Annular corona evident, more than 1 mm long; corolla tube with free corona lobes within . . . . . 4
4. Sepals without visible veins, apices reflexed or not reflexed; follicles free, but usually united at the tips (at least when young), rarely connate (*P. quinquangularis* (Jacq.) Spreng.) . . . . . *Prestonia* sect. *Haemadictyon* (Lindl.) Baill.
4′. Sepals with veins conspicuously impressed, apices not reflexed; follicles usually connate longitudinally . . . . . 5
5. Corolla lobes 18–34 mm long, tube 20–36 mm long . . . *Prestonia* sect. *Mollis* J. F. Morales, M. E. Endress & Liede
5′. Corolla lobes 6–14 mm long, tube 9–7 mm long . . . . . *Prestonia* sect. *Exsertae* J. F. Morales, M. E. Endress & Liede

**Prestonia** sect. **Coalitae** Woodson, Ann. Missouri Bot. Gard. 23: 278. 1936. *Prestonia* sect. *Rhaptocarpus* Pichon, Mém. Mus. Natl. Hist. Nat., B, Bot. 1: 27. 1951, nom. illeg. TYPE: *Prestonia coalita* (Vell.) Woodson, Ann. Missouri Bot. Gard. 18: 552. 1931. *Echites coalitus* Vell., Fl. Flumin., 112. 1825 [1829].

*Prestonia coalita* (Vell.) Woodson, Ann. Missouri Bot. Gard. 18: 552. 1931.

*Prestonia dusenii* (Malme) Woodson, Ann. Missouri Bot. Gard. 18: 552. 1931.

*Prestonia ornata* (Hoehne) J. F. Morales, M. E. Endress & Liede, Taxon 66: 639. 2017.

*Prestonia pickelii* Markgr., Notizbl. Bot. Gart. Berlin-Dahlem 14: 129. 1938.

*Prestonia solanifolia* (Müll. Arg.) Woodson, Ann. Missouri Bot. Gard. 23: 282. 1936.

**Prestonia** sect. **Denticulatae** J. F. Morales, M. E. Endress & Liede, sect. nov. TYPE: *Prestonia denticulata* (Vell.) Woodson, Ann. Missouri Bot. Gard. 23: 284. 1936. *Echites denticulatus* Vell., Fl. Flumin., 110. 1829.

*Prestonia amabilis* J. F. Morales, Sida 21: 161, fig. 1. 2004.

*Prestonia amazonica* (Benth. ex Müll. Arg.) J. F. Macbr., Publ. Field Mus. Nat. Hist., Bot. Ser. 11: 34. 1931.

*Prestonia antioquiana* J. F. Morales & Liede, Phytotaxa 265: 207, figs. 2A, 3A; fig. 4. 2016.

*Prestonia denticulata* (Vell.) Woodson, Ann. Missouri Bot. Gard. 23: 284. 1936.

*Prestonia haughtii* Woodson, Ann. Missouri Bot. Gard. 35: 235. 1948.

*Prestonia leco* A. Fuentes & J. F. Morales, Novon 20: 278, fig. 1. 2010.

*Prestonia lenticellata* A. H. Gentry, Ann. Missouri Bot. Gard. 61: 896. 1974.

*Prestonia lindleyana* Woodson, Bull. Torrey Bot. Club 60: 392. 1933.

*Prestonia macroneura* Woodson, Ann. Missouri Bot. Gard. 23: 321. 1936.

*Prestonia megagros* (Vell.) Woodson, Ann. Missouri Bot. Gard. 21: 623. 1934.

*Prestonia plumierifolia* Markgr., Notizbl. Bot. Gart. Berlin-Dahlem 10: 1038. 1930.

*Prestonia robusta* Rusby, Descr. S. Amer. Pl., 91. 1920.
*Prestonia rotundifolia* K. Schum. ex Woodson, Ann. Missouri Bot. Gard. 23: 318. 1936.
*Prestonia trifida* (Poepp.) Woodson, Bull. Torrey Bot. Club 60: 392. 1933.
*Prestonia vana* Woodson, Ann. Missouri Bot. Gard. 23: 323. 1936.

*Diagnosis.* This section is recognized by its small intrapetiolar colleters, coriaceous to subcoriaceous leaf blades with the secondary veins impressed on both surfaces, coriaceous to subcoriaceous sepals with obscure veins, corolla tube 9–21 mm long with free corona lobes included or apically exserted, well-developed annular corona, and glabrous to puberulent follicles, which are free or that remain longitudinally fused together up to maturity.

**Prestonia** sect. **Exsertae** J. F. Morales, M. E. Endress & Liede, sect. nov. TYPE: *Prestonia exserta* (A. DC.) Standl., J. Wash. Acad. Sci. 15: 460. 1925. *Haemadictyon exsertum* A. DC., Prodr. 8: 426. 1844.

*Prestonia didyma* (Vell.) Woodson, Ann. Missouri Bot. Gard. 23: 308. 1936.
*Prestonia exserta* (A. DC.) Standl., J. Wash. Acad. Sci. 15: 460. 1925.
*Prestonia falcatosepala* J. F. Morales, Candollea 59: 162, fig. 3. 2004.
*Prestonia folsomii* J. F. Morales, Novon 6: 285, fig. 1. 1996.
*Prestonia parvifolia* K. Schum. ex Woodson, Ann. Missouri Bot. Gard. 23: 302. 1936.

*Diagnosis. Prestonia* sect. *Exsertae* is characterized by its diminutive intrapetiolar colleters, membranaceous leaf blades, foliaceous or subfoliaceous sepals with distinct veins, corolla tube 9–17 mm long with exserted or barely included free corona lobes, conspicuous annular corona, and puberulent follicles that are free or that remain longitudinally fused together up to maturity.

**Prestonia** sect. **Haemadictyon** (Lindl.) Baill., Hist. Pl. (Baillon) 10: 203. 1891. *Prestonia* sect. *Haemadictyon* (Lindl.) K. Schum., Nat. Pflanzenfam. 4: 188. 1895, nom. illeg. *Prestonia* sect. *Acutifoliae* Woodson, Ann. Missouri Bot. Gard. 23: 284. 1936, nom. illeg. TYPE: *Prestonia acutifolia* (Benth. ex Müll. Arg.) K. Schum., Nat. Pflanzenfam. 4: 188. 1895. *Haemadictyon acutifolium* Benth. ex Müll. Arg., Fl. Bras. 6: 167. 1860.

*Prestonia* sect. *Annulares* Woodson, Ann. Missouri Bot. Gard. 23: 296. 1936. TYPE: *Prestonia annularis* (L. f.) G. Don, Gen. Hist. 4: 84. 1837. *Echites annularis* L. f., Suppl. Pl., 166. 1781 [1782].

*Prestonia annularis* (L. f.) G. Don, Gen. Hist. 4: 84. 1837.
*Prestonia boliviana* J. F. Morales & A. Fuentes, Sida 21: 166, fig. 1. 2004.
*Prestonia cayennense* (A. DC.) Pichon, Mém. Mus. Natl. Hist. Nat., B, Bot. 1: 25. 1951.
*Prestonia cyaniphylla* (Rusby) Woodson, Ann. Missouri Bot. Gard. 23: 284. 1936.
*Prestonia lagoensis* (Müll. Arg.) Woodson, Ann. Missouri Bot. Gard. 23: 293. 1936.
*Prestonia longifolia* (Sessé & Moc.) J. F. Morales, Novon 6: 287. 1996.
*Prestonia marginata* Markgr., Notizbl. Bot. Gart. Berlin-Dahlem 9: 88. 1924.
*Prestonia papillosa* (Müll. Arg.) J. F. Morales, Candollea 59: 161. 2004.
*Prestonia portobellensis* (Beurl.) Woodson, Ann. Missouri Bot. Gard. 18: 553. 1931.
*Prestonia quinquangularis* (Jacq.) Spreng., Syst. Veg. [Sprengel] 1: 637. 1825 [1824].
*Prestonia vaupesana* Woodson, Bot. Mus. Leafl. 18: 178. 1958.

**Prestonia** sect. **Mollis** J. F. Morales, M. E. Endress & Liede, sect. nov. TYPE: *Prestonia mollis* Kunth, Nov. Gen. Sp. (quarto ed.) 3: 221, pl. 242. 1818 [1819].

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*Diagnosis.* This section is distinguished by its very small intrapetiolar colleters, membranaceous leaf blades, sepals with evident veins, corolla, tube 20–36 mm long with partially exserted free corona lobes, conspicuous annular corona, and glabrous or glabrescent follicles, which remain longitudinally united up to maturity.

**Prestonia** R. Br. sect. **Prestonia.** *Prestonia* sect. *Euprestonia* Baill. Hist. Pl. (Baillon) 10: 203. 1891, nom. inval. *Prestonia* sect. *Tomentosae* Woodson, Ann. Missouri Bot. Gard. 23: 344. 1936, nom. illeg. TYPE: *Prestonia tomentosa* R. Br., Asclepiadeae 59. 1810. (preprint of Mem. Wern. Nat. Hist. Soc. 1: 70. 1811.)

*Prestonia* sect. *Tetraceras* Pichon, Mém. Mus. Natl. Hist. Nat., B, Bot. 1: 26. TYPE: *Prestonia parviflora* (Benth.) Benth. & Hook. f., Gen. Pl. 2: 709. 1876.

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Appendix 1. Voucher data and GenBank accession numbers (*rpl16* intron, *rps16* intron, *trnK* intron, *matK*, *trnL* intron + *trnL-trnF* intergenic spacer, ITS). Asterisk (*) indicates sequences newly generated in this study and a dash (–) missing sequences.

*Artia balansae* Pichon ex Guillaumin, New Caledonia, *G. McPherson 19257* (MO), EF456481, KY794489*, KY524036, EF456325, KY523908, KY483883; *A. brachycarpa* Boiteau, New Caledonia, *H. McKee 4206* (L), KY523983, KY794490*, KY524066, KY524148, KY523909, KY483884; *Asketanthera calycosa* (A. Rich.) Woodson, Cuba, *L. Figueiras 197* (CR), KY523984, –, KY524067, KY524146, KY523910, KY483885.

*Echites tuxtlensis* Standl., Mexico, *J. Soto 22616* (CR), KY523990, KY794491*, KY524070, KY524118, KY523918, KY483893; *E. woodsonianus* Monach., Costa Rica, *J. F. Morales 21413* (CR), EF456403, KY794492*, KY524040, KY524172, KY523919, KY483895.

*Laubertia* sp. nov., Brazil, *G. Prance 26432* (CR), KY524032, KY861242*, KY524112, KY524113, KY523981, KY484002.

*Odontadenia glauca* Woodson, Colombia, *L. Munoz s.n.* (CR), KY524001, KY794493*, KY524081, KY524134, KY523933, KY483909; *O. hypoglauca* (Stadelm.) Müll. Arg., Brazil, *T. Cavalcanti 1066* (CR), KY524002, KY794494*, KY524082, KY524160, KY523934, KY483910.

*Parsonsia crebriflora* Baill., New Caledonia, *G. McPherson 18220* (MO), EF456482, KY794495*, KY524042, KY524161, KY523935, KY483911; *P. ferruginea* J. B. Williams, Australia, *B. Hyland 16142* (Z), EF456486, KY794496*, KY524044, KY524183, KY523937, KY483913; *P. lenticellata* C. T. White, Australia, *P. Forster 28701* (Z), KY524004, KY794497*, KY524083, KY524131, KY523938, KY483914; *P. longiflora* Guillaumin, New Caledonia, *A. Guillaumin 7243* (Z), EF456483, KY794498*, KY524047, KY524164, KY523939, KY483915; *Prestonia acrensis* J. F. Morales, Peru, *T. Croat 85856* (USF), KY794455*, KY794499*, KY861244*, KY861278*, KY794421*, KY829034*; *P. amabilis* J. F. Morales, Ecuador, *H. Lugo 5118* (USF), KY794456*, KY794500*, KY861245*, KY861290*, KY794422*, KY829035*; *P. annularis* (L. f.) G. Don, Bolivia, *R. Seidel 7708* (CR), KY794457*, KY794501*, KY861246*, KY861286*, KY794423*, KY829036*; *P. antioquiana* J. F. Morales & Liede, Colombia, *L. Muñoz s.n.* (CR), KY524013, KY794502*, KY524092, KY524149, KY523951, KY483928; *P. bahiensis* Müll. Arg., Brazil, *F. R. Souza-Silva 211* (HUEFS), KY524014, KY794503*, KY524093, KY524150, KY523952, KY483929; *P. boliviana* J. F. Morales & A. Fuentes, Bolivia, *A. Carretero 994* (CR), KY794458*, KY794504*, KY861247*, KY861307*, KY794424*, KY829037*; *P. calycina* Müll. Arg., Brazil, *G. Siqueira 593* (CR), KY524015, KY794505*, KY524094, KY524154, KY523953, KY483930; *P. cayennense* (A. DC.) Pichon, cult., Costa Rica, *J. F. Morales 21398* (CR), KY794459*, KY794506*, KY861248*, KY861308*, KY794425, KY829038*; *P. clandestina* J. F. Morales, Mexico, *R. Cedillo 3656* (USF), KY794460*, KY794507*, KY861249*, KY861279*, KY794426*, KY829039*; *P. coalita* (Vell.) Woodson, Brazil, *V. Demuner 2433* (CR), EF456447, KY794508*, KY524052, KY524186, KY523954, KY483931; *P. cordifolia* Woodson, cult., Germany, *J. F. Morales s.n.* (CR), KY794461*, KY794509*, KY861250*, KY861285*, KY794427*, –; *P. cyaniphylla* (Rusby) Woodson, Bolivia, *A. Araujo 2604* (CR), KY794462*, KY794510*, KY861251*, KY861309*, KY794428*, KY829040*; *P. denticulata* (Vell.) Woodson, Brazil, *J. Braga 7365* (CR), KY794463*, KY794511*, KY861252*, KY861291*, KY794429*, KY829041*; *P. didyma* (Vell.) Woodson, Brazil, *V. Demuner 2021* (CR), KY794464*, KY794512*, KY861253*, KY861301*, KY794430*, KY829042*; *P. dusenii* (Malme) Woodson, Brazil, *G. Hatschbach 13458* (USF), KY794465*, KY794513*, KY861254*, KY861302*, KY794431*, KY829043*; *P. exserta* (A. DC.) Standl., Panama, *J. Folsom 6899* (MO), KY524016, KY794514*, KY524095, KY524145, KY523955, KY483932; *P. falcatosepala* J. F. Morales, Ecuador, *A. Gentry 70178* (USF), KY794466*, KY794515*, KY861255*, KY861303*, KY794432*, KY829044*; *P. hammelii* J. F. Morales, Costa Rica, *M. Mayfield 1595* (CR), KY794467*, KY794516*, KY861256*, KY861287*, KY794433*, KY829045*; *P. ipomaeifolia* A. DC., Panama, *J. Duke 4885* (CR), KY524017, KY794517*, KY524096, KY524155, KY523956, KY483933; *P. lagoensis* (Müll. Arg.) Woodson, Bolivia, *M. Nee 54002* (USF), KY794468*, KY794518*, KY861257*, KY861310*, KY794434*, KY829046*; *P. leco* A. Fuentes & J. F. Morales, Bolivia, *A. Fuentes 11052* (CR), KY794469*, KY794519*, KY861258*, KY861292*, KY794435*, KY829047*; *P. lenticellata* A. H. Gentry, Panama, *L. Muñoz s.n.* (CR), KY524018, KY794520*, KY524097, KY524151, KY523957, KY483934; *P. lindleyana* Woodson, Brazil, *J. Costa 691* (CR), KY794470*, KY794521*, KY861259*, KY861293*, KY794436*, KY829048*; *P. longifolia* (Sessé & Moc.) J. F. Morales, Costa Rica, *J. F. Morales 21414* (CR), KY794471*, KY794522*, KY861260*, KY861304*, KY794437*, KY829049*; *P. marginata* Markgr., cult., Costa Rica, *J. F. Morales 21505* (CR), KY794472*, KY794523*, KY861261*, KY861288*, KY794438*, KY829050*; *P. mexicana* A. DC., Costa Rica, *J. F. Morales 21447* (CR), EF456487, KY794524*, KY524053, KY524187, KY523958, KY483935; *P. mollis* Kunth, cult., Costa Rica, *J. F. Morales 21422* (CR), KY524019, KY794525*, KY524098, KY524136, KY523959, –; *P. mucronata* Rusby, Colombia, *R. Romero-Castañeda 7892* (CR), KY794473*, KY794526*, KY861262*, KY861299*, KY794439*, KY829051*; *P. ornata* (Hoehne), J. F. Morales & M. E. Endress, Bolivia, *J. Solomon 7679* (CR), KY524025, KY861243*, KY524103, KY524137, KY523973, KY483950; *P. papillosa* (Müll. Arg.) J. F. Morales, Colombia, *L. Muñoz s.n.* (CR), KY794474*, KY794527*, KY861263*, KY861305*, KY794440*, KY829052*; *P. parviflora* (Benth.) Benth. & Hook. f., Peru, *E. Rodriguez 1932* (CR), KY794475*, KY794529*, KY861264*, KY861300*, KY794441*, KY829053*; *P. parvifolia* K. Schum ex Woodson, Ecuador, *L. Escobar 926* (MO), KY524020, KY794528*, KY524099, KY524137, KY523960, KY483937; *P. pickelii* Markgr., Brazil, *L. Silva s.n.* (CR), KY794476*, KY794530*, KY861265*, KY861306*, KY794442*, KY829054; *P. plumierifolia* Markgr.,

Bolivia, *T. Killeen 3606* (USF), KY794477*, KY794531*, KY861266*, KY861294*, KY794443*, KY829055*; *P. portobellensis* (Beurl.) Woodson, Costa Rica, *J. F. Morales 21455* (CR), KY794478*, KY794532*, KY861267*, KY861284*, KY794444*, KY829056*; *P. premontana* J. F. Morales, Venezuela, *I. González s.n.* (CR), KY794479*, KY794533*, KY861268*, KY861295*, KY794445*, KY829057*; *P. quinquangularis* (Jacq.) Spreng., Costa Rica, *J. F. Morales 21419* (CR), KY794480*, KY794534*, KY861269*, KY861289*, KY794446*, KY829058*; *P. riverae* J. F. Morales, Costa Rica, *J. F. Morales 21435* (CR), KY794481*, KY794535*, KY861270*, KY861296*, KY794447*, KY829059; *P. robusta* Rusby, Bolivia, *I. Guareco 264* (CR), EF456463, KY794536*, KY524055, KY524188, KY523962, KY483939; *P. rotundifolia* K. Schum. ex Woodson, Ecuador, *B. Hansen 7995* (USF), KY524022, KY794537*, KY524100, KY524152, KY523963, KY483940; *P. schumanniana* Woodson, Ecuador, *C. Dodson 9982* (USF), KY794482*, KY794538*, KY861271*, KY861280*, KY794448*, KY829060*; *P. seemannii* Miers, Panama, *R. Gonzalez s.n.* (CR), KY794483*, KY794539*, KY861272*, KY861281*, KY794449*, KY829061*; *P. solanifolia* (Müll. Arg.) Woodson, Brazil, *M. Vieira 640* (CR), KY524023, KY794540*, KY524101, KY524132, KY523964, KY483941; *P. speciosa* Donn. Sm., El Salvador, *E. Sandoval 1586* (LAGU), KY794484*, KY794541*, KY861273*, KY861282*, KY794450*, KY829062*; *P. surinamensis* Müll. Arg., Suriname, *R. Evans 3463* (CR), KY794485*, KY794542*, KY861274*, KY861283*, KY794451*, KY829063*; *P. tomentosa* R. Br., Paraguay, *E. Zardini 53321* (CR), EF456484, KY794543*, KY524056, KY524189, KY523965, KY483942; *P. trifida* (Poepp.) Woodson, Costa Rica, *J. F. Morales 21512* (CR), KY524024, KY794544*, KY524102, KY524153, KY523966, KY483943; *P. tysonii* A. H. Gentry, Panama, *L. Muñoz s.n.* (CR), KY794486*, KY794545*, KY861275*, KY861297*, KY794452*, KY829064*; *P. vana* Woodson, Ecuador, *R. Burnham 1767* (CR), KY794487*, KY794546*, KY861276*, KY861298*, KY794453*, KY829065*; *P. vaupesana* Woodson, Venezuela, *R. Liesner 18407* (CR), KY794488*, KY794547*, KY861277*, KY861311*, KY794454*, KY829066*.

*Temnadenia odorifera* (Vell.) J. F. Morales, Brazil, *I. Koch s.n.* (UEC), EF456459, KY794548*, KY524062, KY524191, KY523972, KY483949.